Fabrication of a Picoliter Microreactor

with Multilayer Elastomer Valves

by

Emily Smith

Submitted to the Department of Mechanical
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of Requirements for the Degree of

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ABSTRACT

Microfluidics has the ability to greatly reduce the time needed to do many biological tests. The development of polymers has brought about substrates with elastomeric properties that can be used to the advantage of microfluidic device design. Elastomeric polymers can be used to create small scale passive valve systems. These valves can compartmentalize reactions in devices. Current microreactors only allow researchers to do one test at a time. Devices with the capability to compartmentalize reactions using valves could perform multiple reactions simultaneously. This thesis details the fabrication and design of a microreactor that can maintain cells in the device after a reaction. The fabrication of the device was done without a clean room and using no specialized equipment. Creation of the device and using it for testing requires little training and takes less time than performing the test using conventional methods. The device could readily be made and used by researchers using equipment already in their lab and is cheaper than current devices on the market.

Thesis Supervisor: Todd Thorsen
Title: Assistant Professor of Mechanical Engineering

Acknowledgments

I would like to thank the people who work in the MIT Hatsopoulos Microfluids Laboratory. They were very friendly and helpful and were kind enough to share their lab space.

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Most importantly I would like to thank my thesis advisor Todd Thorsen. I appreciate him giving me an opportunity to learn about the vast field of microfluidics. I enjoyed being able to do biomedical engineering. I also enjoyed him letting me loose on a problem and having faith in me to figure it out.

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1 Introduction

Microfluidics is the manipulations of fluids and gases on the scale of 10-100μm [1]. Manipulation of fluids on a small scale has many applications in the chemical, biological, and medical fields. Configurations of devices can be made to reduce the amount of material needed to for testing, allow for new tests, and reduce the time scale for test. Reduction of scale results in smaller amounts of harmful materials being necessary for a test and smaller amounts of harmful byproducts. New tests can be developed as the size and time-scale becomes feasible. Time-scale can be reduced by allowing researchers to do multiple tests at once and reducing the diffusion time.

1.1 Objective

Biological research involves many tests of a repetitive nature. Old microfluidic devices were made out materials too rigid to make multiple tests on one chip productive. The development of polymers has brought about the creation microfluidic devices out of elastomers. The flexibility of elastomeric substances allows for the creation of passive small scale valves that can compartmentalize testing [2].

The goal of this thesis is to fabricate a microreactor that can contain multiple reactions at the same time and maintain the cells during the reaction period. The methods used to manufacture the device should be inexpensive and not require clean room conditions. The device fabrication process needs to take less time then current manufacture

technology, while minimizing production training time. The following sections explain the reasoning behind design and fabrication decisions in an effort to realize this goal.

2 Current Devices

Current devices that screen toxins are microarrays and microreactors. These devices work by inputting both a toxin and cell line in one end of a long channel and using the low Reynolds number to mix the two substances by diffusion. Biological reactions have Reynolds numbers on the order of .1-1 [3]. The size of channels in microreactors results in laminar flow with Reynolds numbers necessary for these reactions; Reynolds number theory will be discussed later in Section 6.

2.1 Current Microreactor Designs

Microreactors are typically made up of long thin channels and are only capable of performing on reaction at a time, Figure 2.1 [4].

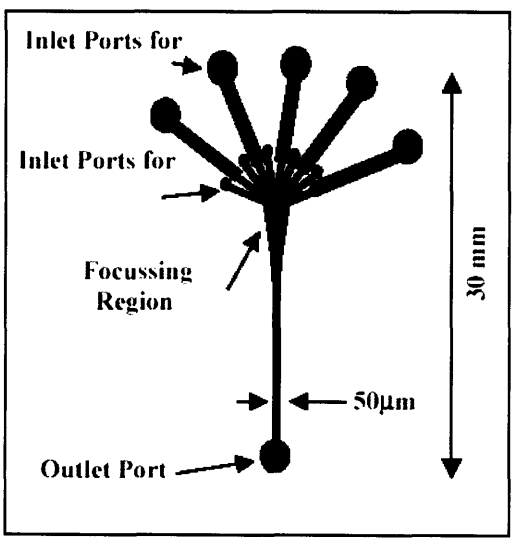

Figure 2.1: Liquid phase microreactor with focusing region, Floyd *et al.* [4].

The device is made out of etched silicon and is capped by glass to forms channels. The long channel in the device shortens the length scale of the diffusion process allowing for fast reactions [4]. Manufacture of the device requires a clean room to etch the channels making the device unrealistic for a typical researchers use. The device also can only do one reaction at a time and would take more time to manufacture than to perform current testing.

Another device that works as a microreactor developed by Leclerc *et al* using polydimethylsiloxane (PDMS) is shown in Figure 2.2 [5].

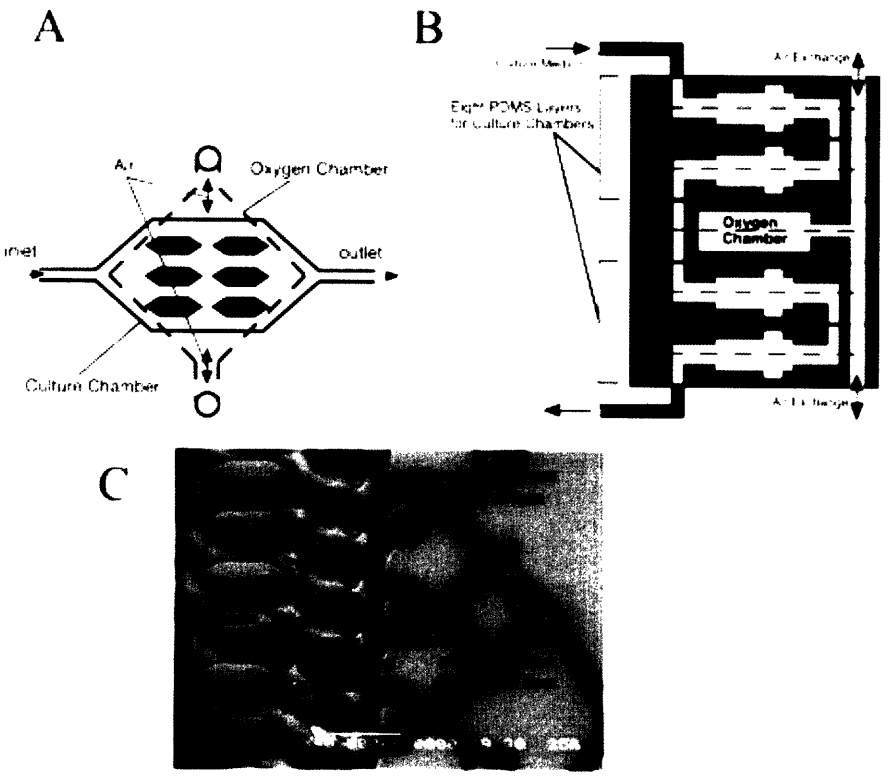

Figure 2.2: Microreactor with oxygen chamber developed by Leclerc *et al.* A) Top-view of oxygen chamber. B) Cross-sectional side view of the device showing the multiple layers of PDMS. C) Schematic of the culture chamber [5].

This device is made out of ten layers of 300μm thick PDMS. The microreactor chambers are made two layers of PDMS stuck together and in between the eight layers is an oxygen chamber. The layers of PDMS used were so thick that an oxygen chamber was necessary to improve cell growth [5]. This device can perform reactions with cells and maintain cell growth but the device can only perform one reaction at a time.

There are several one layer devices that have been developed but few can do multiple reactions. Devices that can do multiple reactions require removal of the assay for determining results. Few devices can do reactions and maintain cells growth like the device developed by Leclerc *et al*, but this device is limited to one reaction at a time.

3 Device Design

The chosen device design allows 32 cell lines to be tested against 32 different toxin lines in the space of a square inch. The number of channels was determined by the number that could optimally fit within a square inch, Figure 3.1.

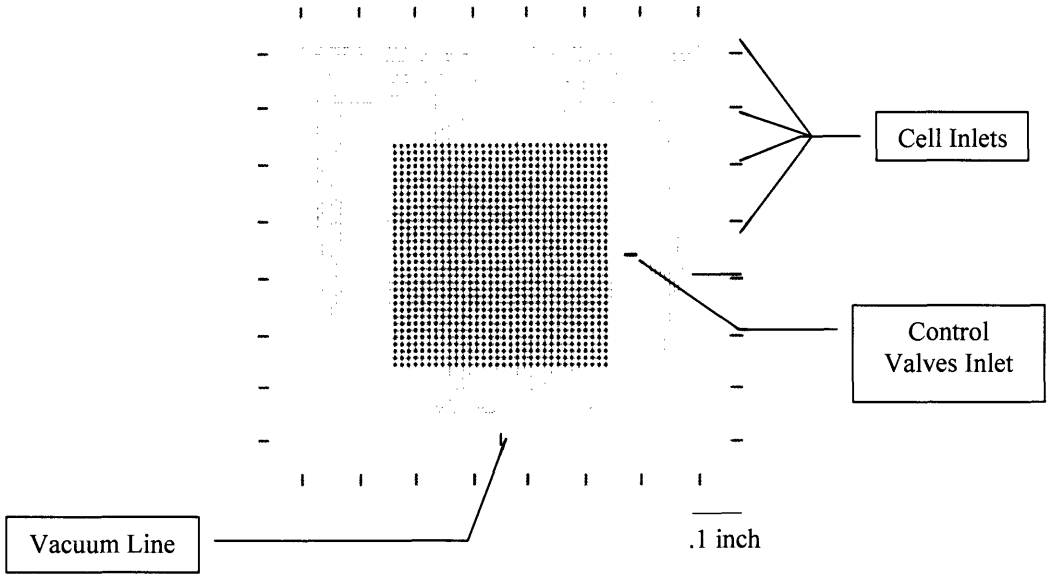

Figure 3.1: Layout of cell channels and control valves and location of bored holes for inlets and the vacuum line.

The cell input lines are shown in Figure 3.2 and measure 100μm wide and vary in length from 2.3 to 3.9cm. The flow channels are mirrored about the vacuum hole.

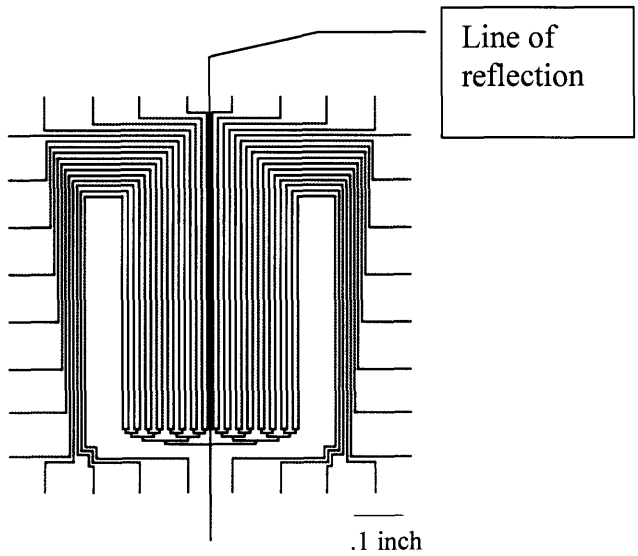

Figure 3.2: Diagram of the pattern used to create the cell channels and the line about which the design is mirrored.

All cell channels have the same length from the end of valve region to the vacuum hole. The control channels are 100μm wide as well making the valve region a square. The control channels are all the same length, 1 cm. There are 33 control lines to make 32 chambers to trap cells. The cell chambers are also 100μm squared and all channels are 10μm high making the dead zone of the valves and the chamber size both 100 pL. The chamber size makes trapping of single cells possible.

The design of this device was previously determined by Prof. Thorsen but a working device was never fabricated. Sections 3.2- 3.5 gives background on the design of the device and explains reasoning for not changing the device design to aid fabrication.

3.1 Soft lithography

The first step to developing the device is determining a way to create a device with the desired pattern. Soft lithography is a group of processes nonphotolithographic methods

for replicating a pattern [1]. Nonphotolithographic techniques can produce lateral features between 30nm to 500um [6]. Soft lithography also offers the advantage of rapid prototyping without a cleanroom. A pattern can be quickly embossed onto a master material and the relief of the image to a substrate. The master, stamp, defines where the material will be removed from the substrate making the process subtractive [2]. Techniques for creating masters include photolithography, micromaching, e-beam writing and relief structures [6]. Soft lithography is the best choice for creating the device pattern to meet the goal of not using a cleanroom during the creation of the device.

3.2 Substrate Materials

PDMS was used as a substrate because it has many properties that are advantageous for this application. The most important characteristic is that it is permeable by gasses, allowing oxygen to diffuse to the cells without needing special inlets. The chemical structure of PDMS makes it hydrophobic, Figure 3.3.

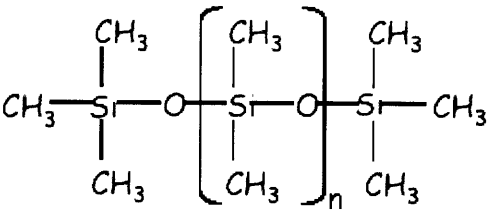

Figure 3.3: Chemical Structure of PDMS showing locations of repeating methyl (CH_3) groups.

The methyl groups make the surface hydrophobic [7]. When water is placed on top of the PDMS it stays in droplet form allowing for testing materials to be directly applied

over the inlet without a need for wells. PDMS is optically clear down to 300nm which could be reduced by adding wells or more thick layers of PDMS [6]. Production of structure with PDMS does not require a clean room and can be used to create minimum features of 10nm [1].

Other materials that are frequently used in microfabrication are silicon and glass. Silicon is much more expensive than PDMS $.05/cm^3 versus $2.5/cm^3 [2] and requires a clean room for processes. Silicon is also difficult to use for making valves because it's young's modulus is about 100GPa [2]. Glass has the same problem of having a high young's modulus that means valves require gaskets and can not be created on the scale required for this device.

Some of the disadvantages of PDMS is elastomers are flexible by nature and can cause unwanted side effects, Figure 3.4 [8].

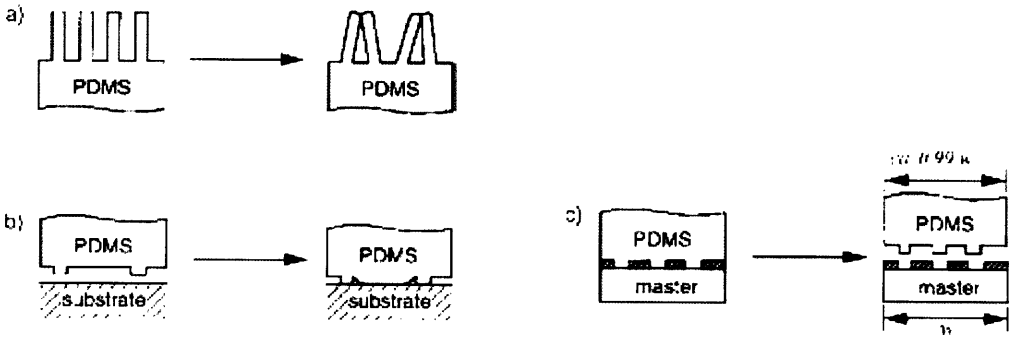

Figure 3.4: Possible problems of design transfer using elastomers. A) Pairing. B) Sagging. C) Shrinking. [8]

The flexible nature of PDMS can cause issues with collapsing and sagging defects in the pattern. PDMS also shrinks ~1% upon curing [6]. All of these defects can be solve for

by designing around them, changing the aspect ratio, adding posts, and expanding layouts on masters. The master pattern needs to have an aspect ratio between .2 and 2 to prevent defects in the stamp [8].

The hydrophobic nature of PDMS can also be a disadvantage because it can prevent cell growth. Cell growth in PDMS is prevented by non-specific protein absorption and binding on the surface [9]. PDMS can be rendered hydrophilic by several methods. Oxygen plasma or functionalized silane treatment is most effective in preventing non-specific absorption and binding or cells and proteins and promoting cell growth [2, 9].

3.3 Valves

The design of the device for toxin screening needs to be small and not require to much extra equipment, therefore a passive valve system must be used since they require the least amount of space. The channels must also be able to hold trap the cells and maintain them long enough to see the effects of the toxins. There are several forms of valves that can trap the cells: microbubbles, thermal expanding microspheres, hydrogels, and multilayer elastomer valves. Microbubbles and thermal expanding microspheres require specialized equipment to make them work and require more training than hydrogels and multilayer valves; therefore they are not good valve choices.

Hydrogels are polymers that change volume when exposed to changing pH [10]. No external control in needed to make them expand and they react only to the environment in the channel [7]. Changing the pH to release or trap cells could be harmful to the cells.

Hydrogels also have a slow reaction times [7], making them a bad choice for a valve system for the desired device properties.

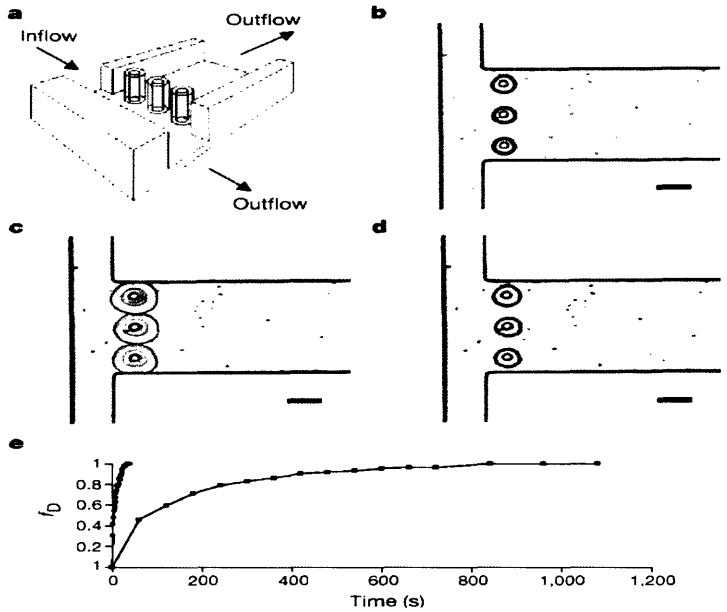

Figure 3.5: Expansion and contraction of hydrogel valves in a T channel. A) Channel layout. B-D) Expansion and contraction of hydrogel valve posts. [10]

Multilayer elastomer valves were created by Unger *et al* [2]. They consist of a passive valve system that works by having a membrane of one channel collapse onto the other.

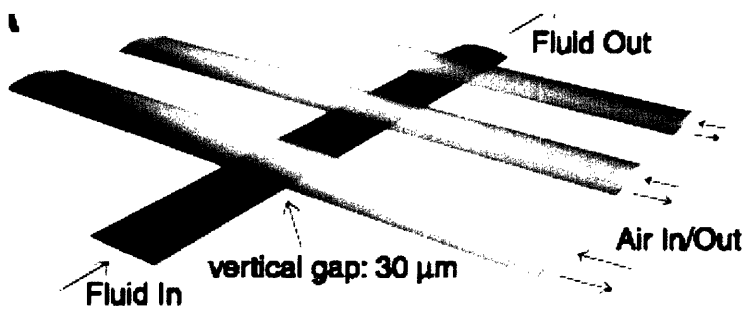

Figure 3.6: Scale diagram of a monolithic multilayer elastomer valve system

Elastomer valves are created when two channels on different layers cross perpendicularly to each other. The steps used to create a valve are shown in Figure 3.7.

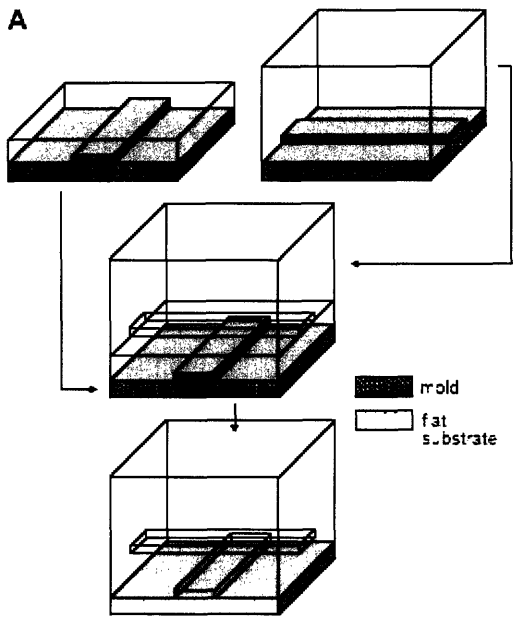

Figure 3.7: Diagram showing steps used to create a multilayer elastomer valve. The two channels are stacked perpendicular to each other creating a thin membrane between the two channels that acts as a valve.

Experimentation by Unger *et al.* found that the thin membrane, 30μm thick, would not close properly for rectangular or trapezoidal valves. Rounded channels were found necessary for complete closure of the channel and will close completely when 40 kPa is applied [2].

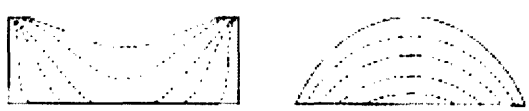

Figure 3.8: Profiles of how valves of the different shapes collapse. The left shows how rectangles leave are only able to make contact in the middle of the channel. [2]

Valves designed by Unger *et al.* have a working area of 100um by 100um, and a reaction times of 1ms when 100kPa of pressure is applied [2]. The small size working size of the valves results in small dead volumes and allows for densely packed devices.

The valve motion follows the Hooke's spring model; they exhibit minimal hystersis [2]. The valves can be opened and close many times with minimal effect on their operation. The valves are also gentle on cells [2]. Elastomer valves are the best choice for the design requirements.

3.4 Fluid Movement

Microfluidics employs many methods to move fluids through devices. Electrokinetic and pressure driven flow are the most common forms of fluid movement. Electrokinetic flow moves molecules by their charge through an electric field. Uniform plug like flow can be created by electrokinetic flow if the frictional forces in the channel are balanced by the electric field [7]. PDMS needs to become negatively charged to support fluid low, which can be done by plasma oxidation. The problem with electrokinetic flow is that it does not work well in bioassays that need uniform flow [7]. The differently charge molecules separate in the electric field.

Pressure driving is the best choice for moving a heterogeneous fluid. Pressure driven flow does have the disadvantage of having a parabolic fluid head. For experimentation needing to trap fluids this can be a problem but in the case of the binary nature of the control of the device the fluid head does not create a problem for the bioassays.

There are two forms of pressure driven flow; pushing with pressure on the inlet or pulling with vacuum on the outlet. Equipment to pressure drive fluid is more readily available and is cheaper than a vacuum pump making it a more accessible choice for driving the flow. The problem is a pushed fluid would not work well if only a few channels were in use. The path of least resistance in this case would be up a channel not in use. The problem could be solved by creating an outlet hole for every inlet, but then the device size would have to change.

The vacuum pulled flow only needs one outlet and will be the path of least resistance for non driven fluid. The hydrophobic nature of PDMS can be used to create droplets over the fluid inlets removing the need for another layer of PDMS for a reservoir, Figure 3.9.

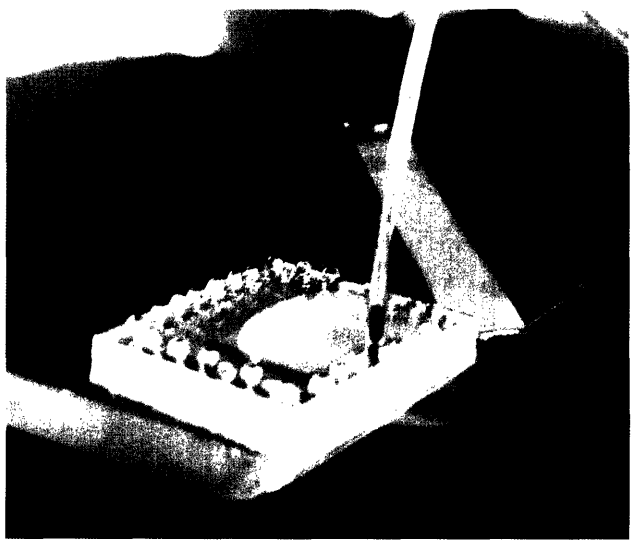

Figure 3.9: Experimental setup showing the vacuum pump line and the droplets used to hold testing material over the inlets.

4 Master Fabrication

Photolithography and soft lithography techniques, when combined, result a in low cost, easy to learn techniques for creating masters. Transparencies printed on a commercial printer with 5080dpi resolution can be used to create a minimum feature size of 20μm [3].

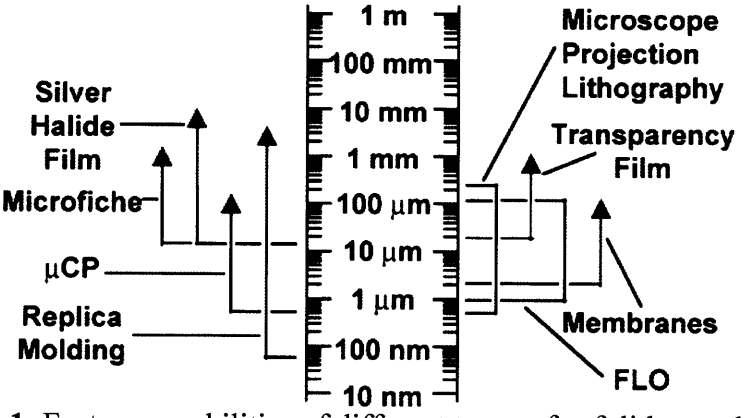

Figure 4.1: Feature capabilities of different types of soft lithography techniques.

The basic steps of creating a master with photolithography are shown in Figure 4.2. A photoresist is spun coat unto a wafer and patterned by exposed areas chemically changing under UV light. The excess photoresist is then removed by developer. The photoresist then becomes even more cross-linked by baking after developing.

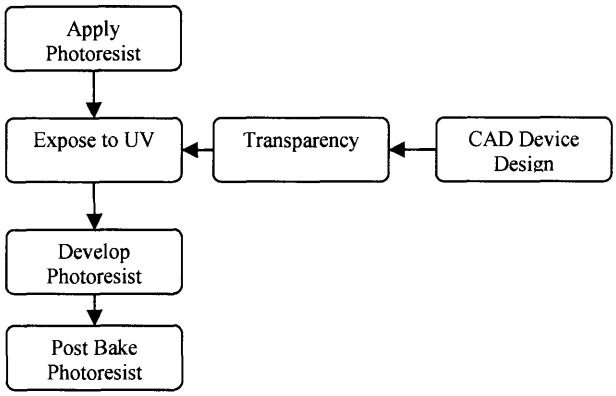

Figure 4.2: Steps to create a master mold using photolithography.

4.1 Photoresist

There are multitudes of photoresist that can be used but only a couple meet the needs of microfluidic devices. The two photoresists that are easiest two use to create molds for PDMS are SU-8 and AZ4620 [11]. The choice of which photoresist is particularly important to getting the necessary wear out of a master and the right feature properties.

Table 1: Material properties of SU-8 and AZ4620

Properties	SU-8	AZ4620
Exposure Type	Negative	Positive
Reflowable	No	Yes

AZ4620 is a positive resist, unexposed resist remains, which means transparencies are easy to print and align. Photomasks for SU-8 must be designed and position so they reach all the way to the edge or a lip of photoresist will be created. Any additive

irregularities to the surface of the thin layer wafer causes problems with getting the desired thickness of PDMS when spin coating.

SU-8 is much more resilient and durable than AZ4620. Wafers are more likely to break than the resist. The hardness of SU-8 is actually the problem for creating the necessary molds. Once exposed to UV light SU-8 becomes so hard that it can not be reflowed, while AZ4620 can be heated for 60 seconds at 150°C to form rounded channel patterns. AZ4620 is the right resist for creating molds with the right features, wide channels with a high aspect ratio and parabolic profile allowing for easy valve closure, for this device.

5 Device Fabrication

The process for the creating of the device is a continuous process that takes approximately three hours to yield all four devices off the wafers. The steps of the process are shown below in Figure 5.1 and a more detailed description can be found in Appendix 2.

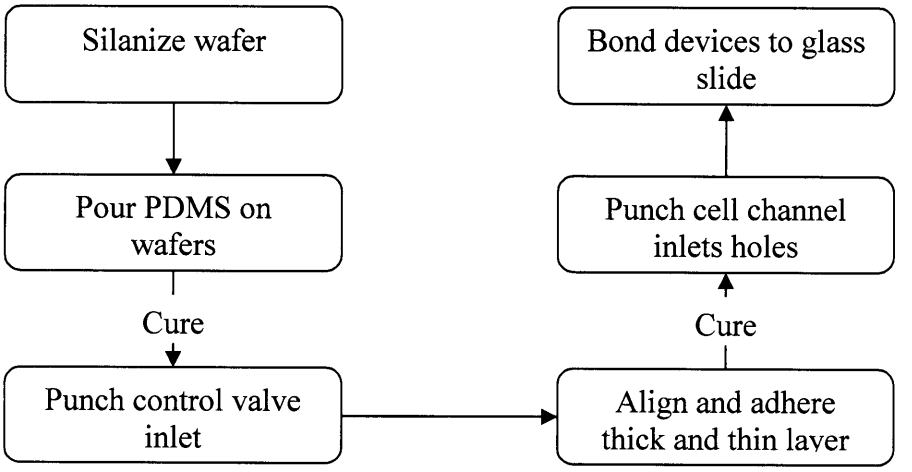

Figure 5.1: Process for fabrication of PDMS device with multilayer elastomer valves.

The device is silanized only the first five times that it is used. The method and silanization process is discussed further in Section 5.1. A 5mm thick layer of PDMS is poured onto the thick layer. The thin layer is spun coat about 30μm thick with PDMS. The thick layer is cured for 18 minutes and the thin layer is cured for 16 minutes at 80°C. The control valve inlets are punched and the four devices on the thick layer are cut from each other. Each device is then individually trimmed, aligned and adhered to the thin layer; method for adhering the two layers to prevent delamination is discussed further in Section 5.3. The devices are then cured for 28 minutes at 80°C. The devices are then cut and peeled from the thin layer wafer. Holes were bored for the channels inlets using a punch guide, effects of the punch guide are discussed in Section 5.4. Finally, the channels are closed of by bonding the device to a glass cover slip. Methods for bonding the device to glass are discussed further in Section 5.5.

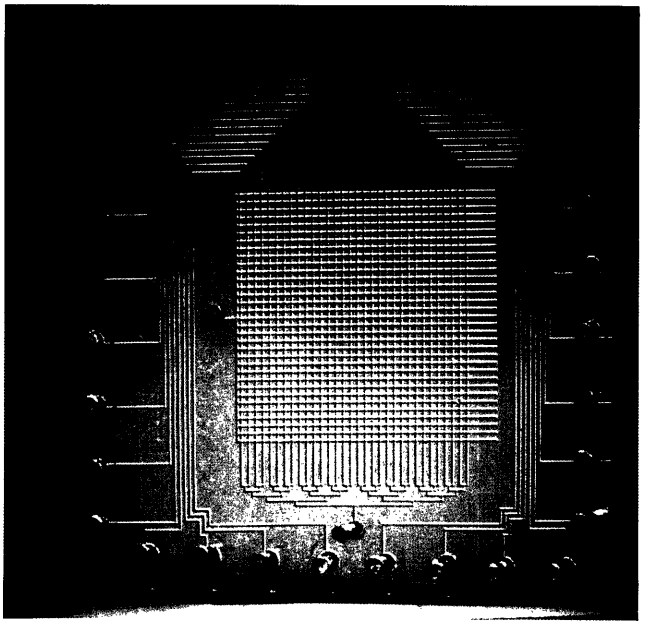

Figure 5.2: Final device sealed to a PDMS coated glass slide with valves open.

5.1 Silanization Method

Silanization is a process of coating the wafer in cholor-trimethyl-silane (silane) to prevent adhesion of the PDMS to the wafer during curing. The silane makes the photoresist less chemically active and reduces the chance that the PDMS will bond irreversibly to the photoresist. Failure to silanize can ruin the master mold.

Silanization can either be done by spin coating or by vapor treatment. Spin coating involves dropping silane directly on the wafer at spinning the wafer at about 4000rpm. Spin coating insures a thin all over layer of silane. The problem with spin coating is that the layer can be more then one layer of silane thick. Only one layer of silane can react to the surface of the wafer while the rest of the silane sits on the wafer and mixes with the PDMS and can cause delamination problems.

Vapor depositing is a self limiting reaction. The wafer is placed in a large covered Petri dish with a few drops of silane. The silane vaporizes and deposits itself everywhere inside the Petri dish including on the wafer. The problem is that the wafer may not be coated evenly but the process is self limiting. The silane will not deposit itself into large layers during the few minutes or hours it is left to silanize.

The process of silanization is only done the first five times the wafer is used. After the wafer has been silanized several times the photoresist can not be changed further by the silanization process. Further silanization was found not to aid release of the thin layer from the wafer and prevent delamination of the two layers of PDMS, instead it may

actually cause delamination of the two layers of PDMS by introducing more contaminates between the layers.

5.2 PDMS Brand

Two common forms of PDMS used in making microfluidic devices are Sylgard 184 and GE RTV615A. The valves developed by Chou *et al* [13] used GE showing that this brand of PDMS can be used for developing monolithic elastomer valves.

During determination of the fabrication process it was found that GE is less likely to delaminate because it is a harder PDMS. The shore hardness of Sylgard is A40 while GE has a shore hardness of A44 [14, 15]. The harder the PDMS the more pressure need to close the valves. GE has longer cure times but after the 18 minutes cure time used to make the Sylgard devices it was already to stiff. Reducing the cure time would not allow enough time for punching of holes in between steps, so it was decided not to use GE for creation of the microreactor with multilayer elastomer valves.

The shorter cure times of Sylgard leaves enough time to punch holes and make other adjustments to the process. Although delamination was a large problem for Sylgard these problems were eventually solved; how these problems were solved will be discussed further in Section 5.3.

5.3 Delamination

Delamination was one of the biggest problems during device fabrication. There are so many things that can cause delamination. The key to lamination is changing the ratio of elastomer base to hardner in the thick and thin layers. Sylgard 184 comes in a ratio 10:1 elastomer base to hardner by weight, when the two layers of PDMS have different ratios diffusion takes place during curing that causes the layers to bond [2]. Finding the correct ratio for the thick and thin layer and making sure the diffusion process can take place during curing is the how to prevent delamination. A ratio of 20:1 for the thin layer and 5:1 for the thick layer was found to work the best; details on the ratio and curing times can be found in Appendix 2.

A major factor that prevents bonding is oil and dirt that gets on the layers before they are cured together. Gloves are not worn during the process of creating the device because the latex can prevent bonding as well. Before handling a cured thick layer it is highly necessary to wash ones hands. Although this is a simple task, it may not be obvious that oil transferred from your hands onto the PDMS layers can prevent the thick layer from bonding to the thin layer.

Another cause of delamination during the creation of this device is over curing. The process must be done in a continuous process, a delay in one step can result in the over curing of another step and prevent things from sticking. It was found that it was better to delay the creation of the thin layer such that there was a gap of about two minutes time for delays. The thick layer will not over cure while out of the oven in two minutes.

Delaminating can also occur if the thick layer is not place onto the thin layer slow enough. Pressing layers together results in stresses that cause the layers to gap and not bond during curing. The layers must be stuck together one channel at a time to ensure that van der Waal forces bond together the layers so they will remain stuck together during curing.

5.4 Punch Guide

A pattern was created to aid in the punching of the 32 inlet holes. The punching of the inlet whole can be difficult and tedious because of their size and multitude. Using the pattern that was sent to the printers a AutoCAD was used to find the hole locations for use with an easytrack mill. The .066" diameter holes were drilled into 1/4" aluminum stock using 8 steps per hole.

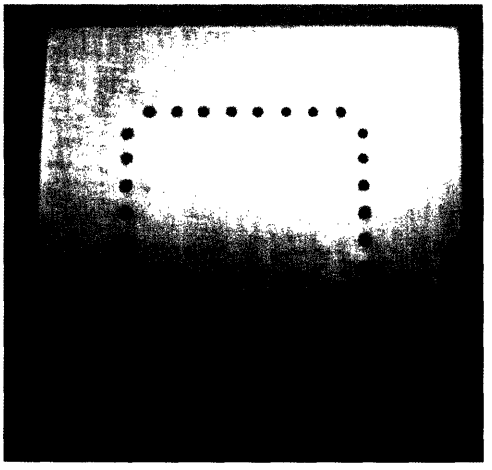

Figure 5.3: Punch guide with device alignment side facing up.

Not only did the hole guide save 15 minutes per chip, but it resulted in perpendicular holes to the top of the chip regardless of the surface used to punch holes on. The easiest way to bore the holes was to place the nonfunctioning side of the device on the a stack of paper towels which allowed enough of the hole center to come away from the surface to easily be removed without touching the device surface. The old method involved pushing the punch twice; once with a hard surface and another into one's finger or other very soft material. The borer also became easier to remove since the PDMS surface was constrained the borer could be removed without struggling with the resistance of the elastomer after it collapsed around the borer. The old method involved repositioning the chip to get tweezers to aid the removal.

Although there were many benefits to the hole punch there were several disadvantages. The van der Waal forces that allow the PDMS to make conformal bonds to glass slides also caused it to bond to the aluminum. The resulting bonding and releasing when pressure was applied by the borer resulted in some chips being damaged by delamination of the bottom layer from the top.

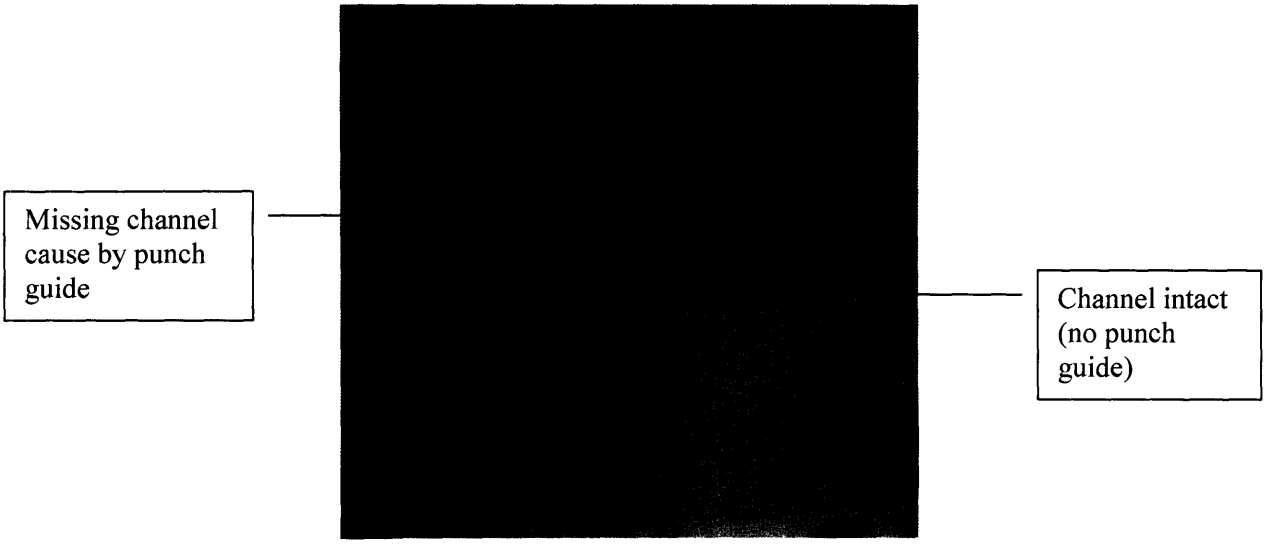

Figure 5.4: Delamination of channel around bored holes caused by the punch guide.

The material choice of aluminum was also a poor once because the borer is stainless steel. The friction created during boring left small metal chips that ended up surrounding the inlets that were punched using the guide.

Figure 5.5: Metal filings left around the punch hole caused by friction between the aluminum borer and aluminum punch guide.

Another issue was making the surface clean enough so that area where the valves were patterned did not get clogged with particles that remained on the aluminum even after cleaning. The debris left behind is clearly shown in Figure 5.4.

Figure 5.6: Particulate left by the punch guide and laminated between the device and PDMS coated slide.

Many of these problems could be solved by choosing to use a different material such as delrin. Delrin has a low friction constant which would prevent the creation of metal chips getting on the device. Delrin also resists bonding to elastomers, like epoxy, which would reduce delamination problems [6]. The clean center surface problem could be taken care of by removing a square of material from the center of the guide. The material in the center is not necessary to support the PDMS during the punching process and is causing the contamination, so it should be removed.

5.5 Channel Closing Method

The cell channels once removed from the wafer are open on the bottom side. PDMS replica molding can only create channels on three sides. The remaining side is to be closed by bonding the PDMS to a glass cover slip. There are three ways to bond the two together: conformal contact, coating the slide in PDMS, or oxygen plasma.

Conformal contact can reversibly seal PDMS to the cover slide using van der Waal forces. This allows for easy cleaning of the device channels that can become clogged with testing. The maximum pressure this type of seal can withstand is 5 psi [7]. During testing the path of least resistance was through the bond between the slide and the device rather than through the channels.

Oxygen plasma was also used bond the device to the slide. Oxygen plasma oxides the exposed face into silanol and the dangling bonds allow the PDMS to bond covalently to the slide [1]. The bond is irreversible and can withstand pressure up to 30 to 50 psi [7]. The device can be bonded anytime after it is cured, but the process requires an asher. This equipment is not readily available to most researchers.

Coating the slide in PDMS was determined to be a third option. One of the advantages to this process is the PDMS would create a strong bond around the control valve inlet, site of many delamination problems. The process requires that the slide be cured the right amount after the holes have been punched into the device. If the slide is under-cured, it will allow the chip to sink into the PDMS and decrease the channel heights. The resulting chip will have a channel resistance too high for testing; channel resistance is discussed further in Section 6.1. When done properly the covalent bond between the slide and the device create a totally sealed device that will withstand delamination. The bond strength of PDMS to PDMS is around 200 psi [16].

5.6 Valve Problems

During punching the device spends most of the time with the channel side up. The membrane that is usually pulled down by gravity into closed position was being pulled up into the control channels. It was found that blowing compressed air or nitrogen into the control inlet could pop the valves up. The valves did not have a problem of curing the slide or to the channel once this process was done. The need for this process precludes the ability to use oxygen plasma because it requires a vacuum that could bond the valves open or closed.

6 Flow Analysis

The Reynolds number, Re, is used to describe the flow of a fluid

$$\mathrm{Re} = \frac{\rho \upsilon D_h}{\mu} \qquad 1$$

where ρ is density of the fluid, υ is the velocity of the fluid, D_h is the hydraulic diameter of the channel and μ is the fluid viscosity [17]. If Re < 2300 than flow within the channel is laminar. Biological reactions like to take place in Reynolds numbers between .1 and 1 [3]. Experimental velocities discussed in Section 6.1 were between $8.8 \cdot 10^{-6}$ and .19 m/s. The range of the Reynolds number for the fluid used of water and fluorescent beads is on the order of 10^{-12} to 10^{-07}, which well within the laminar flow regime.

6.1 Flow velocity

The pressure, ΔP, applied to the channel determines the flow rate, Q, of the fluid, equation 2,

$$Q = \frac{\Delta P}{R} \qquad 2$$

where R is the channel resistance [17]. The channel has a high aspect ratio, the width, w, is 10 times greater than the height, h, so the resistance is,

$$R = \frac{12\mu L}{wh^3} \qquad 3$$

the length of the channel is L [17]. The average velocity, U, is dependent on the flow rate,

$$U = \frac{Q}{wh} \qquad 4$$

The average velocity can be determined by the pressure applied and the channel dimensions. The experimental velocities versus the theoretical velocities are shown in below in Figure 6.1.

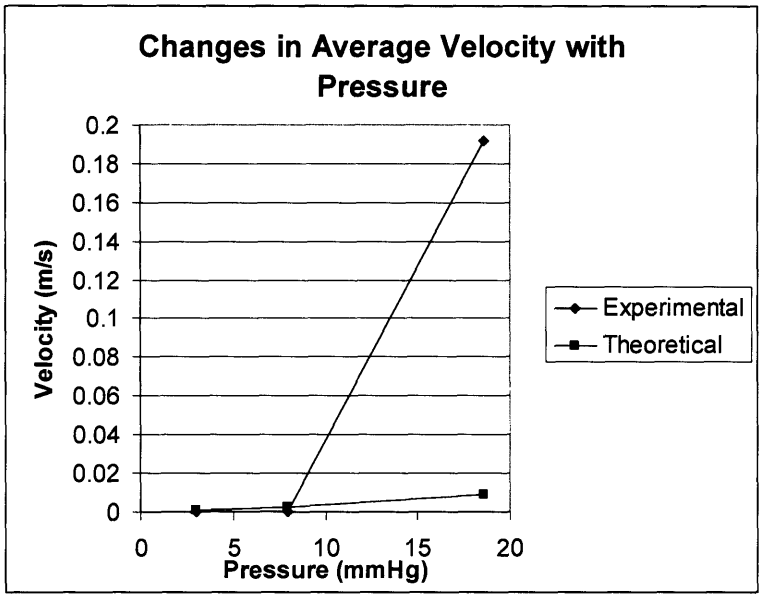

Figure 6.1: Theoretical versus Experimental average velocity at different pressures.

The theoretical pressures and experimental pressures are off by a 100 at 3mmHg and 10 at 7.98mmHg. The cause of this could be differences in designed channel length and experimental channel length. Some of the holes were punch further down on the inlet channel either on accident or there was not enough PDMS to the side of the channel for the punch not to go through the device wall. There are other possible problems of the estimation of the water's density and viscosity while it contains microbeads.

There is also a changing width and height through the control valve region. The control valves add height and width to the channels. Figure 6.2 shows how the fluid head expands and changes while going through valves. The resistance of the channel is increased by every valve.

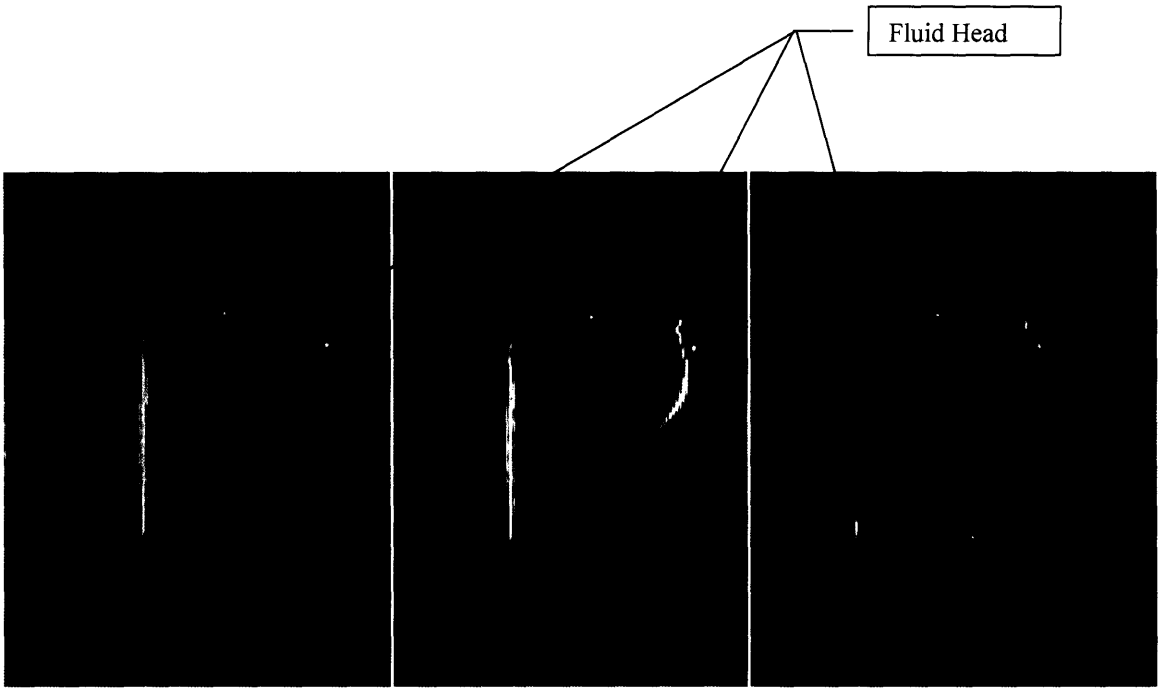

Figure 6.2: Expansion of the fluid head while the fluid is moving through a valve.

The velocity of the high pressure channel was done by computer analysis. Multiple photos of the channel were taken every 5 microseconds. The fluid head location was measured using Adobe Photo Illustrator for each photo. The known width of the channel was used to scale the length in the picture. The multiple estimations and the inaccuracy of the shutter times are potential sources of error in the comparative model.

6.2 Valve Closure

Testing showed that it took around 90 kPa to completely close all the channels when the control lines were full. The control lines were able to close off the cell channels completely. The .51μm beads were unable to move past the valve when it was closed.

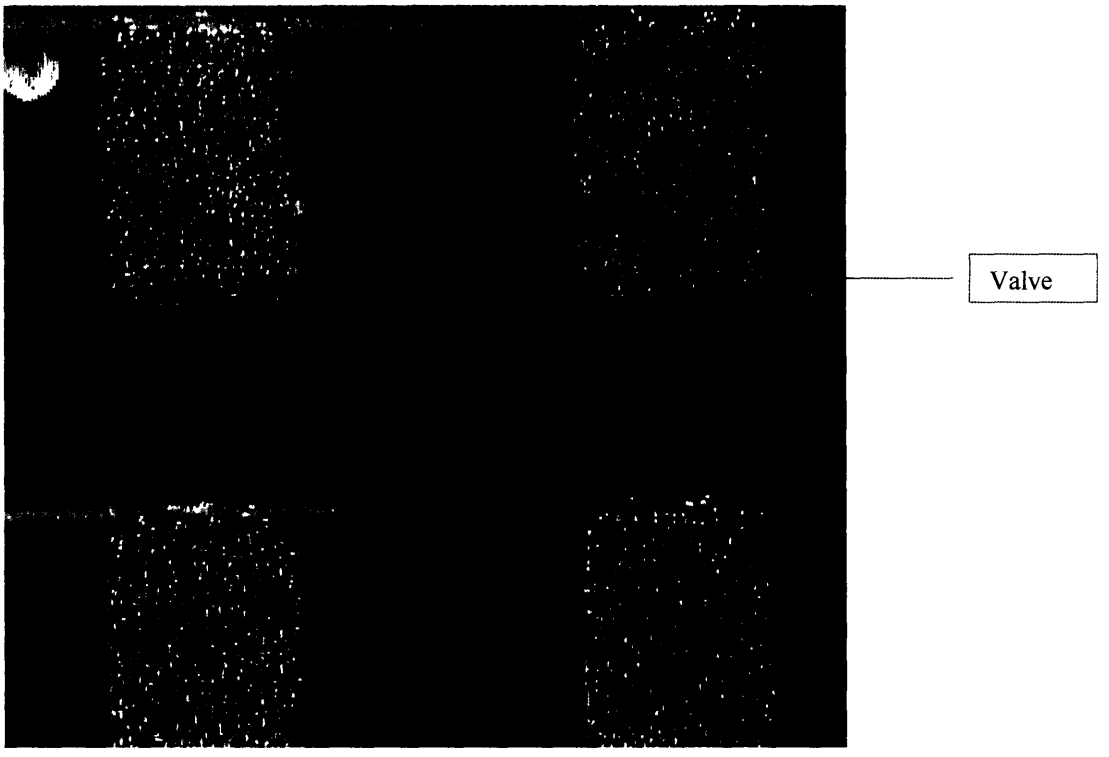

Figure 6.3: .51μm beads trapped by valves when completely closed.

The ability of the valves to trap .51μm beads means that cells could be also trapped in the wells and they would not be able to slip past the valve. The chambers resulting from closing the valves can compartmentalize testing and make results analyze very simple. The contents in each chamber can be looked at under the microscope while still in the device.

6.3 Future Testing

Further testing must be done before the device can be determined to be successful at accomplishing its purpose. The rate of evaporation from the chambers needs to be determined. If fluids evaporate too quickly from the chambers the cells will not be able to survive.

Testing of cell survivability must also be done for the device. The survival rate of cells during flow through the device must be determined first. The survival rate of cells when in chambers must also be determined before toxin testing can be done. A base level of cell survivability must be known before the effects of a toxin can be determined.

7 Conclusion

The fabrication of a multilayer bioreactor with the intent of reacting and maintaining cells is possible. The multilayer elastomer valves are capable of trapping cells into chambers, as shown by the trapping of .51μm beads. The fabrication of this device can be done without a clean room or specialized equipment. There is little training necessary to create the device and it requires less time to build the device than to do tests by current methods. The device could easily be fabricated by researchers in biology, medical or chemical labs. The device fabrication meets the goals laid out in the objectives of this research. Further testing needs to be done to determine the survivability of cells before the device can be used by researchers.

Appendix A- Master Fabrication

A photomasks were created using Adobe Illustrator 11 to drawn designs and printed linotronicly by at 3550 dot per inch onto transparencies (Mika Color, Los Angles, CA). The master fabrication was done in the experimental materials lab (EML) in the MIT Microsystem Technology Lab (MTL) on 3" wafers. Positive photoresist AZ4620 (Clariant) was used to produce a 10μm high master. The wafer is first prepped by spin coating adhesion promoter hexamethyldisilazane (HMDS) at 1000rpm for 60 seconds. A 10μm high layer of photoresist created on the wafer by spin coating at 1500 for 60 seconds.

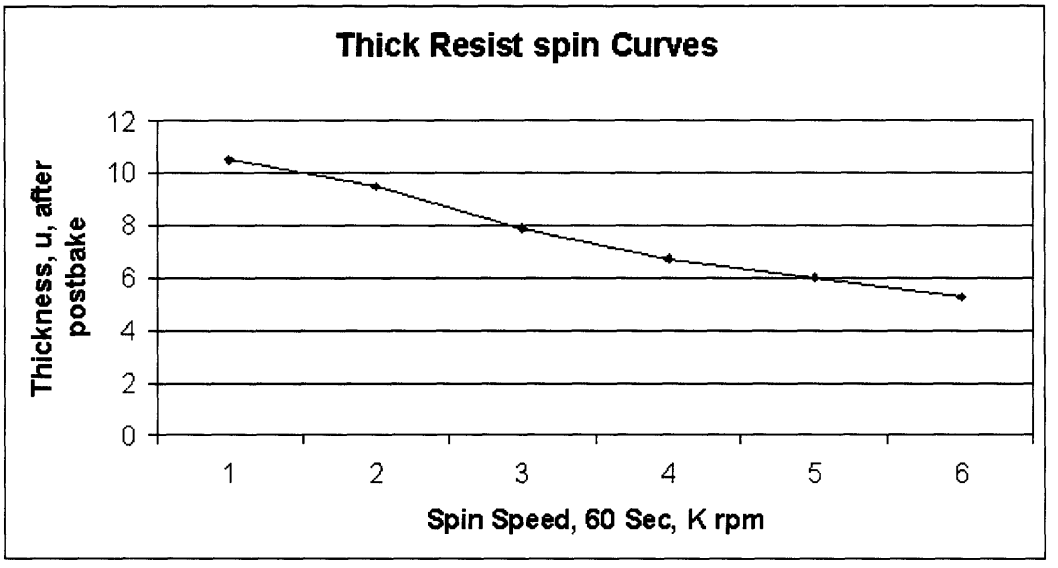

Figure A.1: Spin curve of thickness of AZ4620 for a 60 second spin coat [18].

The photoresist was then prebaked for 20 minutes at 90°C. The contact photolithography was used to expose the photoresist. The transparency was held against the wafer by a

clear quartz plate. Four 16 second burst of 4mW/cm2 UV light was used to expose the photoresist. The developing was in AZ 440 (Clariant).

The photoresist was then reflowed to create the rounded channels necessary for valve closure. Reflowing is done by heating the wafer at 150˚C for 1 minute. The photoresist is then postbaked at 90˚C for 30 minutes.

Appendix B- Device Fabrication

The process of creating the device is a continuous 3 hour process. The first five times the wafer is used it is silanized using vapor depositing methods discussed in Section 5.1. The silanization process was done by placing a few drops of cholor-trimethyl-silane (Aldrich) next to the wafer in a large covered Petri dish. The PDMS silicon elastomer used was Sylgard 184, Section 5.2. The base elastomer is referred to as A and the hardener B by the company. The thick layer was made out of 5 parts A to 1 part B by weight and mixed in a centrifugal mixer and degasser. The 30 grams of liquid elastomer was used when the wafer had not been used previously. Otherwise the wafer was held into the Petri dish by remaining cured elastomer from previous pours and only 15 grams were necessary. The thick layer was poured to about 5mm. The thick layer was then cured at 80°C for 18 minutes. The thin layer was made created by mixing 20 parts A to 1 part B.

The thin layer was mixed and spun coat onto the bottom patter wafer at 3000rpm for 60 seconds. For Sylgard 184 a spin coat at 3000rpm results in a layer of PDMS of 30μm. The thin layer was then baked at 80°C for 16 minutes. The thin layer creation should be done such that thick layer has been peeled from the wafer, split into individual devices and the inlet hole for the control valves bored using a 20 gauge luer stub (Intramedic) before the thin layer is removed. The thin layer can only remain in the oven for 18 minutes or it will not bond to the thick layer. The wafer is ruined if the thin layer does not bond because there is no way to remove the thin layer of PDMS from the stamp pattern without ruining the resist.

The individual thick layers are then slowly adhered unto the thin layer. The layers were aligned with the aid of a dissecting microscope. The dissecting microscope is not necessary to make sure the channels are perpendicular but can aid those not used to aligning the devices. The channels were aligned then one set of corners were place unto the thin layer. The other corners were lowered at the same rate so that the adhesion by van der Waal forces occurred parallel with either the valves or the control channels. During this process the cross sections between the valves adhere individually.

Once the four sectioned pieces of the thick layer are adhered to the thin layer, they are cured in the oven for 28 minutes at 80°C. Slides are then coated in 20A:1B layer of liquid elastomer at 4000rpm for 60 seconds. The 10μm layer of PDMS allows the device to create a bond strong to withstand the pressures needed to use the device. The slides must be made right after the devices go into the oven or they will be too tacky and the device will sink into the PDMS layer decreasing channel height.

The thin layer around each device is then cut to aid in removal of the layer from the wafer. The device is then slowly peeled of the wafer in a manner opposite the way it was applied. Two corners are lifted at the rate of the release of the channels from the wafer. The valves have such thin membranes that the removal must take place parallel to the channels.

It was found that removing the side opposite the inlet valve for the control channels first worked the best. This way all of the channels were released before the inlet valve creates

stresses in the center of removal area. If the inlet channel is peeled first it the valves directly behind it are not peeled at the same time as the rest of valves on the channel resulting in delaminating or weakening of the bond.

The slides should be removed from the oven while the holes are being punched. The device is placed face down on the hole punch guide. The holes of the guide are aligned over channels. The two are flipped over and placed onto paper towels. The paper towels will facilitate the center from the borer coming out of the device, Section 5.4. Each center has to be removed before the borer can be removed or it will pull the center up into the device and compressed so that it can not be removed without repunching and fraying the hole. The vacuum hole is then punched separately after all the holes have been punched.

The punching process causes many of the valves to collapse into the control channels. The valves must be popped back into parallel with the thick layer to prevent membrane from bonding to the device in open position while curing to the slide. The valves can be popped open by blowing compressed air into the control channels. If the bond of the inlet has been weakened by being released before the valves behind it, this process causes these valves to delaminate.

When all the valves are open the device can be adhered to the PDMS coated slide. The method used is the same as for bonding the thick and thin layer together. Place down one side of the device and lower the other side down very slowly allowing the van der Waals

forces to adhere the PDMS together. The bonding process of the device to the PDMS slide is less delicate than the thick to the thin layer because the bond does not have to withstand the forces involved in peeling PDMS of the wafer. The device on the slide is then cured for 1-2 hours at 80°C but can be left in the oven for around 24 hours before the PDMS starts to become too hard and the Petri dish starts to melt.

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